

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
6 May 2004 (06.05.2004)

PCT

(10) International Publication Number
WO 2004/037855 A1

(51) International Patent Classification⁷: **C07K 5/08**,
A61K 38/06

(74) Agent: **BERNIER, Louise, G.**; Boehringer Ingelheim
(Canada) Ltd., 2100 Cunard Street, Laval, Québec H7S
2G5 (CA).

(21) International Application Number:
PCT/CA2003/001604

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE,
GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,
MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT,
RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(22) International Filing Date: 20 October 2003 (20.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/421,414 25 October 2002 (25.10.2002) US
60/433,820 16 December 2002 (16.12.2002) US
60/442,768 27 January 2003 (27.01.2003) US

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*):
BOEHRINGER INGELHEIM INTERNATIONAL
GMBH [DE/DE]; Binger Strasse 173, 55216 Ingelheim
(DE).

Published:

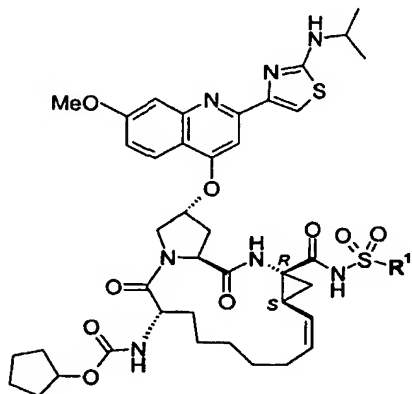
- *with international search report*
- *before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments*

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **LLINAS-BRUNET**,
Montse [CA/CA]; 2100 Cunard Street, Laval, Québec H7S
2G5 (CA). **BAILEY, Murray, D.** [CA/CA]; 2100 Cunard
Street, Laval, Québec H7S 2G5 (CA).

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: MACROCYCLIC PEPTIDES ACTIVE AGAINST THE HEPATITIS C VIRUS



(I)

(57) Abstract: Compounds of formula (I): wherein R¹ is (C₁₋₈)alkyl, (C₃₋₇)cycloalkyl, [(C₁₋₆)alkyl-(C₃₋₇)cycloalkyl] or Het, which are all optionally substituted from 1 to 3 times with halo, cyano, nitro, O-(C₁₋₆)alkyl, amido, amino or phenyl, or R¹ is C₆ or C₁₀ aryl which is optionally substituted from 1 to 3 times with halo, cyano, nitro, (C₁₋₆)alkyl, O-(C₁₋₆)alkyl, amido, amino or phenyl; or a pharmaceutically acceptable salt thereof, useful as an inhibitor of the HCV NS3 protease.

WO 2004/037855 A1

MACROCYCLIC PEPTIDES ACTIVE AGAINST THE HEPATITIS C VIRUS

FIELD OF THE INVENTION

The present invention relates to compounds, processes for their synthesis,
5 compositions and methods for the treatment of hepatitis C virus (HCV) infection. In particular, the present invention provides novel peptide analogs, pharmaceutical compositions containing such analogs and methods for using these analogs in the treatment of HCV infection.

10 BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) is the major etiological agent of post-transfusion and community-acquired non-A non-B hepatitis worldwide. It is estimated that over 200 million people worldwide are infected by the virus. A high percentage of carriers become chronically infected and many progress to chronic liver disease, so-called
15 chronic hepatitis C. This group is in turn at high risk for serious liver disease such as liver cirrhosis, hepatocellular carcinoma and terminal liver disease leading to death.

The mechanism by which HCV establishes viral persistence and causes a high rate of chronic liver disease has not been thoroughly elucidated. It is not known how
20 HCV interacts with and evades the host immune system. In addition, the roles of cellular and humoral immune responses in protection against HCV infection and disease have yet to be established. Immunoglobulins have been reported for prophylaxis of transfusion-associated viral hepatitis, however, the Center for Disease Control does not presently recommend immunoglobulins treatment for this purpose.
25 The lack of an effective protective immune response is hampering the development of a vaccine or adequate post-exposure prophylaxis measures, so in the near-term, hopes are firmly pinned on antiviral interventions.

Various clinical studies have been conducted with the goal of identifying
30 pharmaceutical agents capable of effectively treating HCV infection in patients afflicted with chronic hepatitis C. These studies have involved the use of interferon-alpha, alone and in combination with other antiviral agents. Such studies have shown that a substantial number of the participants do not respond to these therapies, and of those that do respond favorably, a large proportion were found to
35 relapse after termination of treatment.

Until recently, interferon (IFN) was the only available therapy of proven benefit approved in the clinic for patients with chronic hepatitis C. However the sustained response rate is low, and interferon treatment also induces severe side-effects (i.e. retinopathy, thyroiditis, acute pancreatitis, depression) that diminish the quality of life of treated patients. Recently, interferon in combination with ribavirin has been approved for patients non-responsive to IFN alone. However, the side effects caused by IFN are not alleviated with this combination therapy. Pegylated forms of interferons such as PEG-Intron® and Pegasys® can apparently partially address these deleterious side-effects but antiviral drugs still remain the avenue of choice for oral treatment of HCV.

Therefore, a need exists for the development of effective antiviral agents for treatment of HCV infection that overcome the limitations of existing pharmaceutical therapies.

HCV is an enveloped positive strand RNA virus in the Flaviviridae family. The single strand HCV RNA genome is approximately 9500 nucleotides in length and has a single open reading frame (ORF) encoding a single large polyprotein of about 3000 amino acids. In infected cells, this polyprotein is cleaved at multiple sites by cellular and viral proteases to produce the structural and non-structural (NS) proteins. In the case of HCV, the generation of mature nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) is effected by two viral proteases. The first one, as yet poorly characterized, cleaves at the NS2-NS3 junction; the second one is a serine protease contained within the N-terminal region of NS3 (henceforth referred to as NS3 protease) and mediates all the subsequent cleavages downstream of NS3, both in *cis*, at the NS3-NS4A cleavage site, and in *trans*, for the remaining NS4A-NS4B, NS4B-NS5A, NS5A-NS5B sites. The NS4A protein appears to serve multiple functions, acting as a cofactor for the NS3 protease and possibly assisting in the membrane localization of NS3 and other viral replicase components. The complex formation of the NS3 protease with NS4A seems necessary to the processing events, enhancing the proteolytic efficiency at all of the sites. The NS3 protein also exhibits nucleoside triphosphatase and RNA helicase activities. NS5B is a RNA-dependent RNA polymerase that is involved in the replication of HCV.

35

A general strategy for the development of antiviral agents is to inactivate virally encoded enzymes that are essential for the replication of the virus.

5 The following is a list of patent application published in the last few years that disclose HCV NS3 protease inhibitor peptide analogs that are structurally different from the compounds of the present invention:

WO 98/17679 (published April 30, 1998); WO 99/50230 (published October 7, 1999); WO 01/74768 (published October 11, 2001); WO 98/22496 (published May
10 28, 1998); US 6,187,905; WO 97/43310 (published November 20, 1997); WO 01/58929 (published August 16, 2001); WO 01/77113 (published October 18, 2001); WO 01/81325 (published November 1, 2001); WO 98/46597 (published October 22, 1998); WO 98/46630 (published October 22, 1998); JP 10298151 (published November 10, 1998); JP 11127861 (published May 18, 1999); JP 2001103993
15 (published April 17, 2001); JP 11292840 (published October 26, 1999); WO 99/38888 (published August 5, 1999); WO 99/64442 (published December 16, 1999); WO 00/31129 (published June 2, 2000); WO 01/32691 (published May 10, 2001); US 6,159,938 (published December 12, 2000); WO 01/02424 (published January 11, 2001); WO 01/07407 (published February 1, 2001); WO 01/40262
20 (published June 7, 2001); and WO 01/64678 (published September 7, 2001).

Peptide analogs which inhibit the HCV NS3 protease have been disclosed in WO 00/09543 (published February 24, 2000), WO 00/09558 (published February 24, 2002), WO 00/59929 (published October 12, 2000) and WO 02/060926 (published
25 August 8, 2002). The compounds of the present invention distinguish themselves by having a different chemical structure and by the surprising finding that they specifically inhibit HCV NS3 protease while showing insignificant inhibitory activity against other serine proteases. The WO 00/59929 discloses the corresponding terminal acid of the present compounds, which also exhibit specificity. WO
30 03/053349 published on July 3, 2003 also discloses macrocyclic tripeptide inhibitors of hepatitis C virus. Nevertheless, the specific activity of the present compounds was unexpected.

One advantage of the present invention is that it provides tripeptide compounds that
35 are inhibitory to the NS3 protease, an enzyme essential for the replication of the

hepatitis C virus.

A further advantage of one aspect of the present invention resides in the fact that the compounds specifically inhibit the NS3 protease and do not show significant

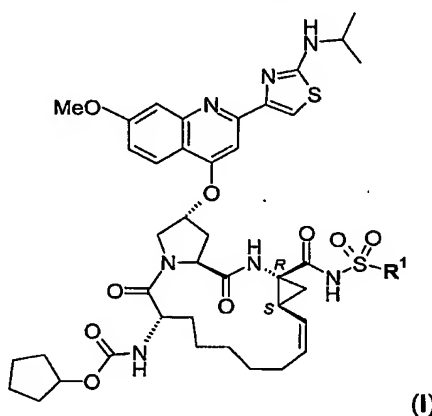
- 5 inhibitory activity against other serine proteases such as human leukocyte elastase (HLE), porcine pancreatic elastase (PPE), or bovine pancreatic chymotrypsin, or cysteine proteases such as human liver cathepsin B (Cat B).

Furthermore, the compounds are active in cell culture.

10

SUMMARY OF THE INVENTION

Included in the scope of the invention are compounds of formula (I):



- 15 wherein R^1 is (C_{1-8}) alkyl, (C_{3-7}) cycloalkyl, $\{(C_{1-6})$ alkyl- (C_{3-7}) cycloalkyl $\}$ or Het, which are all optionally substituted from 1 to 3 times with halo, cyano, nitro, $O-(C_{1-6})$ alkyl, amido, amino or phenyl, or R^1 is C_6 or C_{10} aryl which is optionally substituted from 1 to 3 times with halo, cyano, nitro, (C_{1-6}) alkyl, $O-(C_{1-6})$ alkyl, amido, amino or phenyl; or a pharmaceutically acceptable salt thereof.

- 20 Included within the scope of this invention is a pharmaceutical composition comprising an anti-hepatitis C virally effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent.

- 25 Also within the scope of this invention is the use of a compound of formula I, as described herein, for the manufacture of a medicament for the treatment or

prevention of hepatitis C viral infection.

According to one embodiment, the pharmaceutical composition of this invention further comprises interferon or pegylated-interferon. Alternatively, the composition
5 comprises a compound of formula (I) in combination with Ribavirin. As a further alternative, the composition comprises a compound of formula (I) in combination with interferon (pegylated or not) and ribavirin.

An important aspect involves a method of inhibiting the replication of hepatitis C
10 virus by exposing the virus to a hepatitis C NS3 protease-inhibiting amount of a compound of formula (I), or a pharmaceutically acceptable salt thereof or a composition as described above.

Another important aspect of the invention involves a method of treating or preventing
15 a hepatitis C viral infection in a mammal by administering to the mammal an anti-hepatitis C virally effective amount of a compound of formula I, a pharmaceutically acceptable salt thereof, or a composition as described above, alone or in combination with interferon or ribavirin or both.

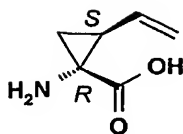
20 DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

As used herein, the following definitions apply unless otherwise noted:
With reference to the instances where (*R*) or (*S*) is used to designate the absolute configuration of a substituent, e.g. R^4 of the compound of formula I, the designation
25 is done in the context of the whole compound and not in the context of the substituent alone.

The designation "P1, P2, and P3" as used herein refer to the position of the amino acid residues starting from the C-terminus end of the peptide analogs and extending
30 towards the N-terminus (i.e. P1 refers to position 1 from the C-terminus, P2: second position from the C-terminus, etc.) (see Berger A. & Schechter I., Transactions of the Royal Society London series B257, 249-264 (1970)).

As used herein the term "(1*R*, 2*S*)-vinyl-ACCA" refers to a compound of formula:



namely, (1*R*, 2*S*) 1-amino-2-ethenylcyclopropylcarboxylic acid.

5 The term "halo" as used herein means a halogen substituent selected from bromo, chloro, fluoro or iodo.

The term "(C₁₋₈)alkyl" as used herein, either alone or in combination with another substituent, means acyclic, straight or branched chain alkyl substituents containing from 1 to 8 carbon atoms and includes, for example, methyl, ethyl, propyl, butyl,
10 hexyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl.

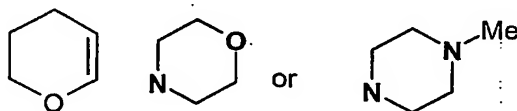
The term "(C₃₋₇)cycloalkyl" as used herein, either alone or in combination with another substituent, means a cycloalkyl substituent containing from three to seven carbon atoms and includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and
15 cycloheptyl.

The term "{(C₁₋₆)alkyl-(C₃₋₇)cycloalkyl}" as used herein means a cycloalkyl radical containing from 3 to 6 carbon atoms directly linked to an alkylene radical containing 1 to 7 carbon atoms; for example, cyclopropylmethyl, cyclopentylethyl,
20 cyclohexylmethyl, and cyclohexylethyl. In the instance where R¹ is a {(C₁₋₆)alkyl-(C₃₋₇)cycloalkyl}, this group is attached to the SO₂ group via the (C₁₋₆)alkyl (i.e. the alkylene portion).

The term "O-(C₁₋₆)alkyl" or "C₁₋₆alkoxy" as used herein interchangeably, either alone
25 or in combination with another substituent, means the substituent -O-(C₁₋₆)alkyl wherein alkyl is as defined above containing up to six carbon atoms. O-(C₁₋₆)alkyl includes methoxy, ethoxy, propoxy, 1-methylethoxy, butoxy and 1,1-dimethylethoxy. The latter substituent is known commonly as *tert*-butoxy.

30 The term "Het" as used herein, either alone or in combination with another substituent, means a monovalent substituent derived by removal of a hydrogen from a five-, six-, or seven-membered saturated or unsaturated (including aromatic)

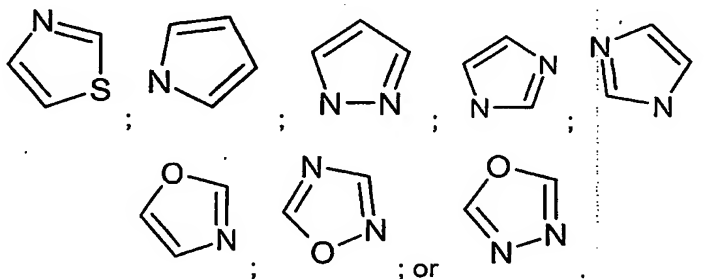
heterocycle containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur. Examples of suitable heterocycles include: tetrahydrofuran, thiophene, diazepine, isoxazole, piperidine, dioxane, pyrimidine or



5

The term "Het " also includes a heterocycle as defined above fused to one or more other cycle be it a heterocycle or any other cycle. One such examples includes thiazolo[4,5-b]-pyridine.

- 10 Although generally covered under the term "Het", the term "heteroaryl" as used herein precisely defines an unsaturated heterocycle for which the double bonds form an aromatic system. Suitable example of heteroaromatic system include: quinoline, indole, pyridine,



15

- The term "pharmaceutically acceptable salt" means a salt of a compound of formula (I) which is, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation,
- 20 allergic response, and the like, commensurate with a reasonable benefit/risk ratio, generally water or oil-soluble or dispersible, and effective for their intended use. The term includes pharmaceutically-acceptable acid addition salts and pharmaceutically-acceptable base addition salts. Lists of suitable salts are found in, e.g., S.M. Birge et al., J. Pharm. Sci., 1977, 66, pp. 1-19, which is hereby incorporated by reference
- 25 in its entirety.

The term "pharmaceutically-acceptable acid addition salt" means those salts which

retain the biological effectiveness and properties of the free bases and which are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, sulfamic acid, nitric acid, phosphoric acid, and the like, and organic acids such as acetic acid, trichloroacetic acid, trifluoroacetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, 2-acetoxybenzoic acid, butyric acid, camphoric acid, camphorsulfonic acid, cinnamic acid, citric acid, digluconic acid, ethanesulfonic acid, glutamic acid, glycolic acid, glycerophosphoric acid, hemisulfic acid, heptanoic acid, hexanoic acid, formic acid, fumaric acid, 2-hydroxyethanesulfonic acid (isethionic acid), lactic acid, maleic acid, hydroxymaleic acid, malic acid, malonic acid, mandelic acid, mesitylenesulfonic acid, methanesulfonic acid, naphthalenesulfonic acid, nicotinic acid, 2-naphthalenesulfonic acid, oxalic acid, pantoic acid, pectinic acid, phenylacetic acid, 3-phenylpropionic acid, picric acid, pivalic acid, propionic acid, pyruvic acid, pyruvic acid, salicylic acid, stearic acid, succinic acid, sulfanilic acid, tartaric acid, p-toluenesulfonic acid, undecanoic acid, and the like.

The term "pharmaceutically-acceptable base addition salt" means those salts which retain the biological effectiveness and properties of the free acids and which are not biologically or otherwise undesirable, formed with inorganic bases such as ammonia or hydroxide, carbonate, or bicarbonate of ammonium or a metal cation such as sodium, potassium, lithium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically-acceptable organic nontoxic bases include salts of primary, secondary, and tertiary amines, quaternary amine compounds, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion-exchange resins, such as methylamine, dimethylamine, trimethylamine, ethylamine, diethylamine, triethylamine, isopropylamine, tripropylamine, tributylamine, ethanolamine, diethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, tetramethylammonium compounds, tetraethylammonium compounds, pyridine, N,N-dimethylaniline, N-methylpiperidine, N-methylmorpholine, dicyclohexylamine, dibenzylamine, N,N-dibenzylphenethylamine, 1-phenamine,

N,N'-dibenzylethylenediamine, polyamine resins, and the like. Particularly preferred organic nontoxic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline, and caffeine.

- 5 The term "antiviral agent" as used herein means an agent (compound or biological) that is effective to inhibit the formation and/or replication of a virus in a mammal. This includes agents that interfere with either host or viral mechanisms necessary for the formation and/or replication of a virus in a mammal. Antiviral agents include, for example, ribavirin, amantadine, VX-497 (merimepodib, Vertex Pharmaceuticals), VX-
10 498 (Vertex Pharmaceuticals), Levovirin, Viramidine, Ceplene (maxamine), XTL-001 and XTL-002 (XTL Biopharmaceuticals).

- The term "immunomodulatory agent" as used herein means those agents (compounds or biologicals) that are effective to enhance or potentiate the immune
15 system response in a mammal. Immunomodulatory agents include, for example, class I interferons (such as α -, β -, δ - and omega interferons, tau-interferons, consensus interferons and asialo-interferons), class II interferons (such as γ -interferons) and pegylated interferons.

- 20 The term "inhibitor of HCV NS3 protease" as used herein means an agent (compound or biological) that is effective to inhibit the function of HCV NS3 protease in a mammal. Inhibitors of HCV NS3 protease include, for example, those compounds described in WO 99/07733, WO 99/07734, WO 00/09558, WO 00/09543, WO 00/59929 or WO 02/060926, and the Vertex pre-development
25 candidate identified as VX-950. Particularly, compounds # 2, 3, 5, 6, 8, 10, 11, 18, 19, 29, 30, 31, 32, 33, 37, 38, 55, 59, 71, 91, 103, 104, 105, 112, 113, 114, 115, 116, 120, 122, 123, 124, 125, 126 and 127 disclosed in the table of pages 224-226 in WO 02/060926, can be used in combination with the compounds of the present invention.

- 30 The term "inhibitor of HCV polymerase" as used herein means an agent (compound or biological) that is effective to inhibit the function of an HCV polymerase in a mammal. This includes, for example, inhibitors of HCV NS5B polymerase. Inhibitors of HCV polymerase include non-nucleosides, for example, those compounds
35 described in:

- WO 03/010140 (Boehringer Ingelheim),
- WO 03/010141 (Boehringer Ingelheim),
- WO 03/007945 (Boehringer Ingelheim),
- WO 03/026587 (Bristol Myers Squibb);
- 5 • WO 02/100846 A1 and WO 02/100851 A2 (both Shire),
- WO 01/85172 A1 and WO 02/098424 A1 (both GSK),
- WO 00/06529 and WO 02/06246 A1 (both Merck),
- WO 01/47883 and WO 03/000254 (both Japan Tobacco) and
- EP 1 256 628 A2 (Agouron).

10

Furthermore other inhibitors of HCV polymerase also include nucleoside analogs, for example, those compounds described in:

- WO 01/90121 A2 (Idenix),
- WO 02/069903 A2 (Biocryst Pharmaceuticals Inc.), and
- 15 • WO 02/057287 A2 and WO 02/057425 A2 (both Merck/Isis).

Specific examples of inhibitors of an HCV polymerase, include JTK-002/003 and JTK-109 (Japan Tobacco), and NM283 from Idenix.

The term "inhibitor of another target in the HCV life cycle" as used herein means an agent (compound or biological) that is effective to inhibit the formation and/or replication of HCV in a mammal other than by inhibiting the function of the HCV NS3 protease. This includes agents that interfere with either host or HCV viral mechanisms necessary for the formation and/or replication of HCV in a mammal. Inhibitors of another target in the HCV life cycle include, for example, agents that inhibit a target selected from a helicase, an HCV NS2/3 protease and an internal ribosome entry site (IRES). Specific examples of inhibitors of another target in the HCV life cycle include ISIS-14803 (ISIS Pharmaceuticals).

The term "HIV inhibitor" as used herein means an agent (compound or biological) that is effective to inhibit the formation and/or replication of HIV in a mammal. This includes agents that interfere with either host or viral mechanisms necessary for the formation and/or replication of HIV in a mammal. HIV inhibitors include, for example, nucleosidic inhibitors, non-nucleosidic inhibitors, protease inhibitors, fusion inhibitors and integrase inhibitors.

The term "HAV inhibitor" as used herein means an agent (compound or biological) that is effective to inhibit the formation and/or replication of HAV in a mammal. This includes agents that interfere with either host or viral mechanisms necessary for the formation and/or replication of HAV in a mammal. HAV inhibitors include Hepatitis A vaccines, for example, Havrix[®] (GlaxoSmithKline), VAQTA[®] (Merck) and Avaxim[®] (Aventis Pasteur).

The term "HBV inhibitor" as used herein means an agent (compound or biological) that is effective to inhibit the formation and/or replication of HBV in a mammal. This includes agents that interfere with either host or viral mechanisms necessary for the formation and/or replication of HBV in a mammal. HBV inhibitors include, for example, agents that inhibit HBV viral DNA polymerase or HBV vaccines. Specific examples of HBV inhibitors include Lamivudine (Epivir-HBV[®]), Adefovir Dipivoxil, Entecavir, FTC (Coviracil[®]), DAPD (DXG), L-FMAU (Clevudine[®]), AM365 (Amrad), Ldt (Telbivudine), monoval-LdC (Valtorecitabine), ACH-126,443 (L-Fd4C) (Achillion), MCC478 (Eli Lilly), Racivir (RCV), Fluoro-L and D nucleosides, Robustaflavone, ICN 2001-3 (ICN), Bam 205 (Novelos), XTL-001 (XTL), Imino-Sugars (Nonyl-DNJ) (Synergy), HepBzyme; and immunomodulator products such as: interferon alpha 2b, HE2000 (Hollis-Eden), Theradigm (Epimmune), EHT899 (Enzo Biochem), Thymosin alpha-1 (Zadaxin[®]), HBV DNA vaccine (PowderJect), HBV DNA vaccine (Jefferon Center), HBV antigen (OraGen), BayHep B[®] (Bayer), Nabi-HB[®] (Nabi) and Anti-hepatitis B (Cangene); and HBV vaccine products such as the following: Engerix B, Recombivax HB, GenHevac B, Hepacare, Bio-Hep B, TwinRix, Comvax, Hexavac.

The term "class I interferon" as used herein means an interferon selected from a group of interferons that all bind to receptor type I. This includes both naturally and synthetically produced class I interferons. Examples of class I interferons include α -, β -, δ -, omega interferons, tau-interferons, consensus interferons, asialo-interferons.

The term "class II interferon" as used herein means an interferon selected from a group of interferons that all bind to receptor type II. Examples of class II interferons include γ -interferons.

The pharmaceutical compositions of the invention may contain one or more additional active agents selected, for example, from antiviral agents, immunomodulatory agents, inhibitors of HCV polymerase, other inhibitors of HCV NS3 protease, inhibitors of another target in the HCV life cycle, HIV inhibitors, HAV inhibitors and HBV inhibitors. Examples of such agents are provided in the Definitions section above.

Specific preferred examples of some of these agents are listed below:

- antiviral agents: ribavirin and amantadine.
- immunomodulatory agents: class I interferons, class II interferons and pegylated interferons.
- inhibitors of HCV polymerase: non-nucleosides and nucleoside analogs.
- inhibitor of another target in the HCV life cycle that inhibits a target selected from: NS3 helicase, HCV NS2/3 protease or internal ribosome entry site (IRES).
- HIV inhibitors: nucleosidic inhibitors, non-nucleosidic inhibitors, protease inhibitors, fusion inhibitors and integrase inhibitors.
- HBV inhibitors: agents that inhibit HBV viral DNA polymerase or is an HBV vaccine.

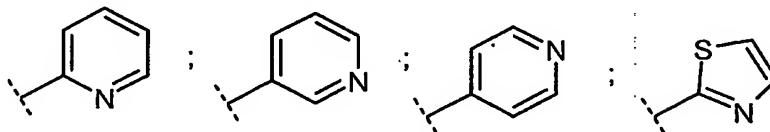
As discussed above, combination therapy is contemplated wherein a compound of formula (I), or a pharmaceutically acceptable salt thereof, is co-administered with at least one additional agent selected from: an antiviral agent, an immunomodulatory agent, an inhibitor of HCV polymerase, another inhibitor of HCV NS3 protease, an inhibitor of another target in the HCV life cycle, an HIV inhibitor, an HAV inhibitor and an HBV inhibitor. Examples of such agents are provided in the Definitions section above. These additional agents may be combined with the compounds of this invention to create a single pharmaceutical dosage form. Alternatively these additional agents may be separately administered to the patient as part of a multiple dosage form, for example, using a kit. Such additional agents may be administered to the patient prior to, concurrently with, or following the administration of a compound of formula (I), or a pharmaceutically acceptable salt thereof.

Preferred embodiments

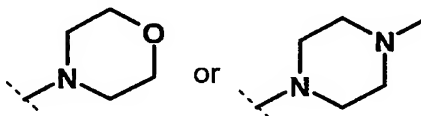
Preferably, a compound of formula I is as defined above wherein R^1 is (C_{1-6}) alkyl,

(C₃₋₆)cycloalkyl, {(C₁₋₆)alkyl-(C₃₋₆)cycloalkyl} or Het, which are all optionally substituted 1 to 3 times with halo, nitro or O-(C₁₋₆)alkyl, or phenyl which is optionally substituted from 1 to 3 times with halo, nitro, (C₁₋₆)alkyl or O-(C₁₋₆)alkyl.

- 5 Preferably, Het is selected from: a heteroaryl selected from:



said heteroaryl optionally substituted with C₁₋₆ alkyl; or
a heterocycle selected from:



10

More preferably, a compound of formula I is as defined above wherein R¹ is (C₁₋₆)alkyl, (C₃₋₆)cycloalkyl, or {(C₁₋₆)alkyl-(C₃₋₆)cycloalkyl}, which are all optionally substituted 1 to 3 times with halo, nitro or O-(C₁₋₆)alkyl, or phenyl which is optionally substituted from 1 to 3 times with halo, nitro, (C₁₋₆)alkyl or O-(C₁₋₆)alkyl.

15

More preferably, R¹ is selected from: methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *sec*-butyl, *n*-pentyl, 2-Me-butyl, 3-Me-butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclopropylmethyl, cyclohexylethyl, CCl₃, CF₃, phenyl, 2-fluorophenyl, or 4-methylphenyl.

20

Even more preferably, R¹ is selected from: methyl, ethyl, *n*-propyl, *i*-propyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclopropylmethyl, cyclohexylethyl, CCl₃, CF₃, phenyl, 2-fluorophenyl, or 4-methylphenyl.

25

Most preferably, R¹ is methyl, cyclopropyl, CF₃ or phenyl. Again most preferably, R¹ is methyl, cyclopropyl or phenyl. Still, most preferably, R¹ is methyl. Alternatively, most preferably, R¹ is cyclopropyl. Again most preferably, R¹ is phenyl.

According to an alternate embodiment, the pharmaceutical composition of this

invention may additionally comprise another anti-HCV agent. Examples of anti-HCV agents include, α - (alpha), β - (beta), δ - (delta), γ - (gamma) or ω - (omega) interferon, ribavirin and amantadine.

- 5 According to another alternate embodiment, the pharmaceutical composition of this invention may additionally comprise an inhibitor of HCV polymerase.

According to another alternate embodiment, the pharmaceutical composition of this invention may additionally comprise another inhibitor of HCV NS3 protease.

10

According to yet another alternate embodiment, the pharmaceutical composition of this invention may additionally comprise an inhibitor of other targets in the HCV life cycle, including but not limited to, helicase, NS2/3 protease or internal ribosome entry site (IRES).

15

The pharmaceutical composition of this invention may be administered orally, parenterally or via an implanted reservoir. Oral administration or administration by injection is preferred. The pharmaceutical composition of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrasternal, intrathecal, and intralesional injection or infusion techniques.

25

The pharmaceutical composition may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example Tween 80) and suspending agents.

30

The pharmaceutical composition of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, and aqueous suspensions and solutions. In the case of tablets for oral use, carriers

35

which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

Other suitable vehicles or carriers for the above noted formulations and compositions can be found in standard pharmaceutical texts, e.g. in "Remington's Pharmaceutical Sciences", The Science and Practice of Pharmacy, 19th Ed. Mack Publishing Company, Easton, Penn., (1995).

Dosage levels of between about 0.01 and about 100mg/kg body weight per day, preferably between about 0.1 and about 50mg/kg body weight per day of the protease inhibitor compound described herein are useful in a monotherapy for the prevention and treatment of HCV mediated disease. Typically, the pharmaceutical composition of this invention will be administered from about 1 to about 5 times per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (w/w). Preferably, such preparations contain from about 20% to about 80% active compound.

As the skilled artisan will appreciate, lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the infection, the patient's disposition to the infection and the judgment of the treating physician. Generally, treatment is initiated with small dosages substantially less than the optimum dose of the peptide. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. In general, the compound is most desirably administered at a concentration level that will generally afford antivirally effective results without causing any harmful or deleterious

side effects.

When the composition of this invention comprise a combination of a compound of formula I and one or more additional therapeutic or prophylactic agent, both the
5 compound and the additional agent should be present at dosage levels of between about 10 to 100%, and more preferably between about 10 and 80% of the dosage normally administered in a monotherapy regimen.

When these compounds or their pharmaceutically acceptable salts are formulated
10 together with a pharmaceutically acceptable carrier, the resulting composition may be administered *in vivo* to mammals, such as man, to inhibit HCV NS3 protease or to treat or prevent HCV virus infection. Such treatment may also be achieved using a compound of this invention in combination with agents which include, but are not limited to: α -, β -, δ -, ω -, or γ -interferon, ribavirin, amantadine; inhibitors of HCV
15 polymerase; other inhibitors of HCV NS3 protease; inhibitors of other targets in the HCV life cycle, which include but not limited to, helicase, NS2/3 protease, or internal ribosome entry site (IRES); or combinations thereof. The additional agents may be combined with compounds of this invention to create a single dosage form. Alternatively these additional agents may be separately administered to a mammal
20 as part of a multiple dosage form.

Accordingly, another embodiment of this invention provides a method of inhibiting HCV NS3 protease activity in a mammal by administering a compound of the formula
I.
25

In a preferred embodiment, this method is useful in decreasing the NS3 protease activity of the hepatitis C virus infecting a mammal.

If the pharmaceutical composition comprises only a compound of this invention as
30 the active component, such method may additionally comprise the step of administering to said mammal an agent selected from an immunomodulatory agent, an antiviral agent, a HCV polymerase inhibitor, a HCV NS3 protease inhibitor, or an inhibitor of other targets in the HCV life cycle such as helicase, NS2/3 protease or IRES. Such additional agent may be administered to the mammal prior to,
35 concurrently with, or following the administration of the composition of this invention.

A compound of formula I set forth herein may also be used as a laboratory reagent. A compound of this invention may also be used to treat or prevent viral contamination of materials and therefore reduce the risk of viral infection of
5 laboratory or medical personnel or patients who come in contact with such materials (e.g. blood, tissue, surgical instruments and garments, laboratory instruments and garments, and blood collection apparatuses and materials).

A compound of formula I set forth herein may also be used as a research reagent. A
10 compound of formula I may also be used as positive control to validate surrogate cell-based assays or *in vitro* or *in vivo* viral replication assays.

Further details of the invention are illustrated in the following examples which are understood to be non-limiting with respect to the appended claims.

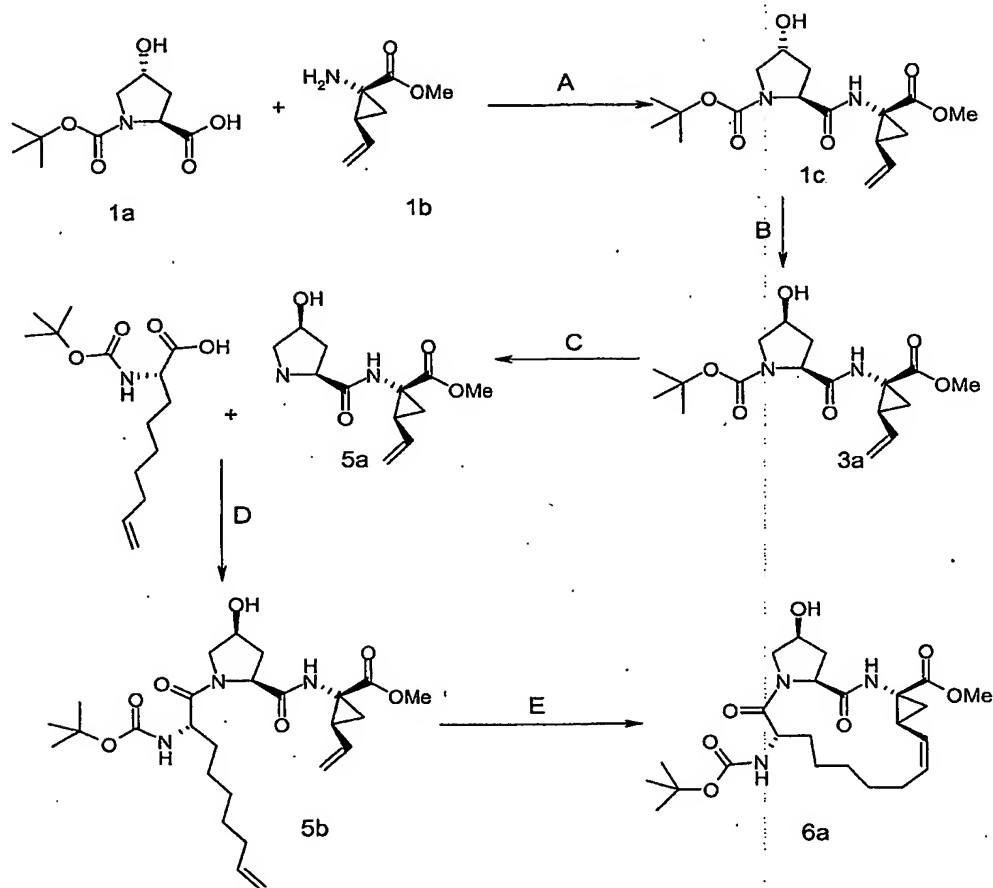
15

Methodology

In general, the compound of formula I and intermediates therefore are prepared by known methods using reaction conditions which are known to be suitable for the reactants. Several such methods are disclosed in WO 00/09543 and WO 00/09558
20 incorporated herein by reference.

The following scheme illustrates a convenient process using known methods for preparing a key intermediate of formula 6a from acyclic intermediates:

SCHEME 1

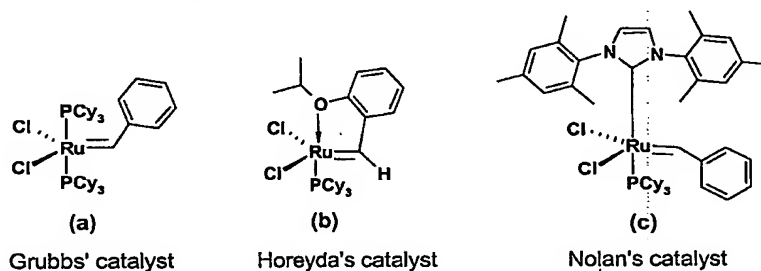


Scheme 1:

There are several ways in which the coupling sequences A and C can be carried out which can be easily recognized by persons skilled in the art. Starting with 4-(S)-hydroxyproline, the substituent at the 4-hydroxy can be incorporated via a Mitsunobu reaction (as described in Mitsunobu *Synthesis* **1981**, January, 1-28; Rano *et al. Tet. Lett.* **1994**, 36, 3779-3792; Krchnak *et al. Tet. Lett.* **1995**, 36, 6193-6196) before or after the macrocyclization. Alternatively the assembly can be done with the required 4-(R)-hydroxy-substituted proline as disclosed in the general processes of WO 00/09543 & WO 00/09558.

Steps A, B, C, D: Briefly, the P1, P2, and P3 moieties can be linked by well known peptide coupling techniques generally disclosed in WO 00/09543 & WO 00/09558.

Step E: The formation of the macrocycle can be carried out via an olefin metathesis using a Ru-based catalyst such as the one reported by Miller, S.J.; Blackwell, H.E.; Grubbs, R.H. *J. Am. Chem. Soc.* 1996, 118, 9606-9614 (a); Kingsbury, J.S.; Harrity, J.P.A.; Bonitatebus, P.J.; Hoveyda, A.H. *J. Am. Chem. Soc.* 1999, 121, 791-799 (b) and Huang, J.; Stevens, E.D.; Nolan, S.P.; Petersen, J.L.; *J. Am. Chem. Soc.* 1999, 121, 2674-2678 (c). It will also be recognized that catalysts containing other transition metals such as Mo can be used for this reaction.



Subsequent conversion of the key intermediate of formula 6a to the compounds of formula 1 of this invention is disclosed in detail in the examples hereinafter.

EXAMPLES

The present invention is illustrated in further detail by the following non-limiting examples. Other specific ways of synthesis or resolution can be found in WO 00/09543 & WO 00/09558.

Temperatures are given in degrees Celsius. Solution percentages express a weight to volume relationship, and solution ratios express a volume to volume relationship, unless stated otherwise. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer; the chemical shifts (δ) are reported in parts per million and are referenced to the internal deuterated solvent unless otherwise indicated. The NMR spectra of all final compounds (inhibitors) was recorded in DMSO-d₆ of their TFA salt unless otherwise indicated. Flash column chromatography was carried out on silica gel (SiO₂) according to Still's flash chromatography technique (W.C. Still et al., *J. Org. Chem.*, 1978, 43, 2923).

Abbreviations used in the examples include vinyl-ACCA: 1-amino-2-vinyl cyclopropylcarboxylic acid; Boc: *tert*-butoxycarbonyl [Me₃COC(O)]; BSA: bovine

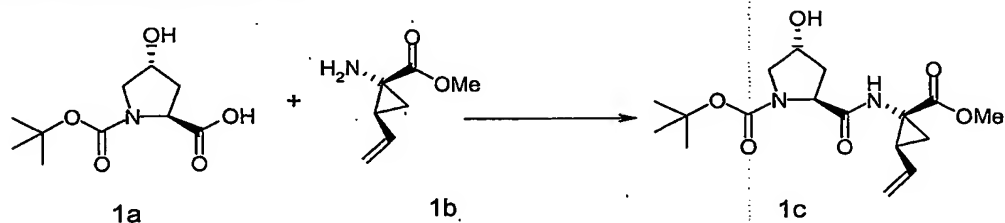
serum albumin; Cbz: benzyloxycarbonyl; CHAPS: 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; DBU: 1,8-Diazabicyclo[5.4.0]undec-7-ene; DCM: CH₂Cl₂; methylene chloride; DEAD: diethylazodicarboxylate; DIAD: diisopropylazodicarboxylate; DIPEA: diisopropylethylamine; DMAP: dimethylaminopyridine; DMF: *N,N*-dimethylformamide; DMSO: dimethylsulfoxide; DPPA: diphenylphosphoryl azide; EDTA: ethylenediamine tetraacetic acid; (*S,S*)- Et-DUPHOS Rh (COD)OTf: (+)-1,2-bis (2*S*,5*S*)-2,5-diethylphospholano) benzene (cyclooctadiene) rhodium (1) trifluoromethanesulfonate; EtOH: ethanol; EtOAc: ethyl acetate; ESMS: electrospray mass spectrometry; eq.: equivalent(s); h.: hour(s);

10 HATU: O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HPLC: high performance liquid chromatography; MS: mass spectrometry; MALDI-TOF: Matrix Assisted Laser Desorption Ionization-Time of Flight; min.: minute(s); FAB: Fast Atom Bombardment; Me: methyl; MeOH: methanol; Ph: phenyl; Pr: propyl; RP-HPLC: reverse phase HPLC; Rt: retention time; RT: room temperature

15 (18° -22°); sat.: saturated; TBTU: 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA: trifluoroacetic acid; THF: tetrahydrofuran; TLC: thin layer chromatography; Tris/HCl: tris(hydroxymethyl)aminomethane hydrochloride.

20 EXAMPLE 1

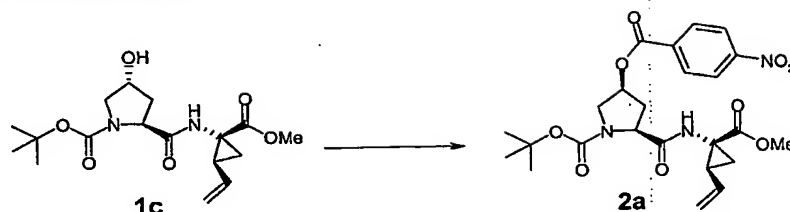
Synthesis of dipeptide **1c**:



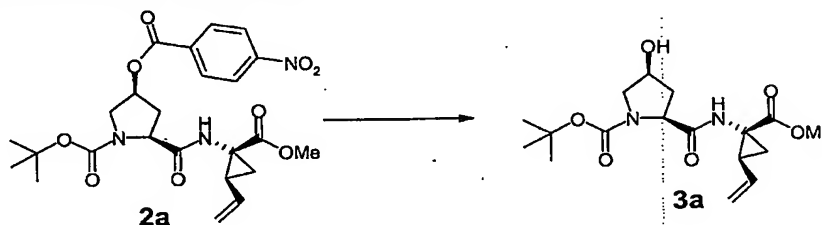
A mixture of Boc-hydroxyproline **1a** (50.0g, 216mmol), (1*R*,2*S*)-vinyl-ACCA hydrochloride **1b** (42.25g, 238mmol), TBTU (76.36g, 238mmol) and DIPEA (113mL, 649mmol) in DMF (800mL) was stirred at R.T. under a nitrogen atmosphere. After

25 3.5h, the solvent was evaporated and the residue extracted with EtOAc and washed with hydrochloric acid (10%), saturated sodium bicarbonate and brine. The organic phase was then dried over magnesium sulfate, filtered and evaporated to afford an oil. Drying the oil overnight (18h) under high vacuum gave the dipeptide **1c** as a

30 yellow foam (72.0 g, 94%, purity >95% by HPLC).

EXAMPLE 2**Synthesis of dipeptide 2a**

5 The dipeptide **1c** (72.0g, 203mmol), triphenylphosphine (63.94g, 243.8mmol, 1.2equiv.) and 4-nitrobenzoic acid (41.08g, 245.8mmol, 1.2equiv.) were dissolved in dry THF (1.4L) and the stirred solution cooled to 0° under a nitrogen atmosphere. DEAD (38.4mL, 244mmol, 1.2equiv.) was then added dropwise over 45 min and the reaction allowed to warm to R.T. After 4h, the solvent was evaporated and the residue divided into four portions. Each of these was chromatographed over fine silica gel (10-40 μ m mesh, column diameter 12 cm, column length 16cm) using a gradient of 2 : 1 hexane/EtOAc to 1:1 hexane/EtOAc to pure EtOAc. Ester **2a** was obtained as an amorphous white solid after evaporation of the solvents and drying under high vacuum at 70° for 1h (108.1g, quantitative yield).

EXAMPLE 3**Synthesis of alcohol dipeptide 3a:**

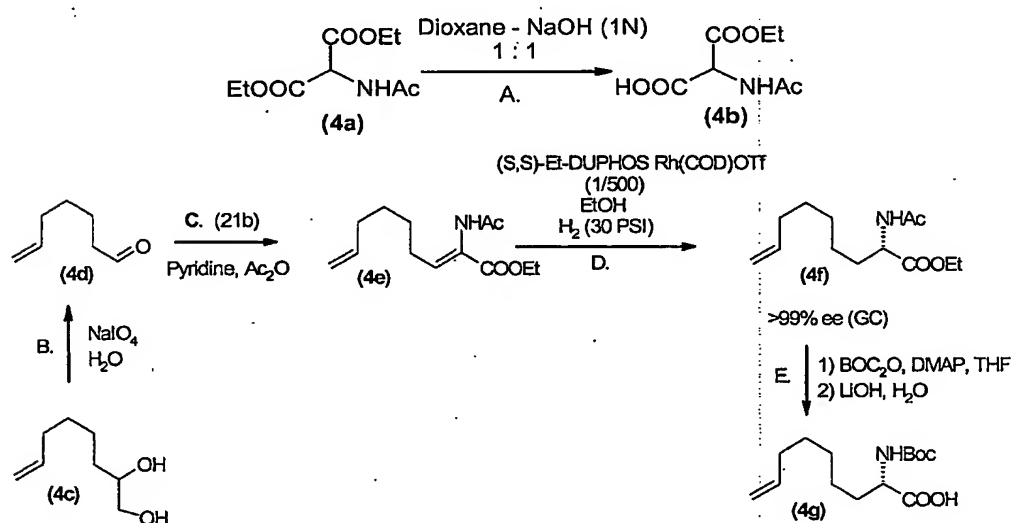
The nitrobenzoyl ester **2a** (108.1g, 203.1mmol) was dissolved in THF (1.0L) and the resulting solution cooled to 0°. A solution of lithium hydroxide monohydrate (10.66g, 253.9mmol) in water (225mL) was then added rapidly and the reaction stirred at 0° for 30 min. after which time the remaining base was neutralized with hydrochloric acid (1N, 50.8mL). Additional acid was slowly added until the yellow color dissipated (7mL). The resulting mixture was then evaporated and the residue extracted with EtOAc (3 x 150mL) and washed with saturated sodium bicarbonate (150mL) and

brine (150mL). The organic phase was dried over magnesium sulfate-charcoal, filtered through diatomaceous earth and evaporated. Overnight drying of the residue under high vacuum yielded the alcohol **3a** as a colorless foam (70.1g, 98%, purity >99% by HPLC).

5

EXAMPLE 4

Synthesis of (2S)-N-Boc-amino-non-8-enoic acid (**4g**)



10

Step A. To a solution of commercially available diethyl 2-acetamidomalonate **4a** (100 g, 0.46mole) in dioxane (500mL) was added aqueous sodium hydroxide (1M, 1eq., 460mL) dropwise over 30 to 45min. The resulting mixture was left to stir for 16.5h, then dioxane was evaporated *in vacuo*. The resulting aqueous solution was extracted with three portions of 300mL of EtOAc and acidified to pH 1 with concentrated HCl. This solution was left to crystallize in an ice-water bath. After the appearance of a few crystals, the mixture was sonicated and an abundant precipitate appeared. Filtration and drying under vacuum afforded compound **4b**, (62.52g, 72% yield) as a white solid.

15

Step B. To a magnetically stirred emulsion of commercially available 7-octene-1,2-diol **4c** (25g, 0.173mole) and H₂O (100mL), in a 1L round bottom flask, an aqueous solution of sodium periodate (40.7g, 0.190moles, 1.1eq., in 475mL H₂O) was added over a period of 20 min (slightly exothermic). The resulting mixture was stirred at room temperature for an additional 1h (completion of reaction confirmed by TLC).

20

The mixture was then decanted in a separatory funnel and the aqueous layer was separated from the organic layer. The aqueous solution was saturated with NaCl, decanted and separated from the organic fraction once more. The two organic fractions were combined, dried with sodium sulfate and filtered over a cotton plug (in
5 a Pasteur pipette) to give compound **4d** (15.135g, colorless oil, 78% yield). The aqueous solution was extracted with CH₂Cl₂, dried with anhydrous MgSO₄, and concentrated under vacuum (without heating, i.e. 6-heptanal b.p.153°C) to obtain an additional amount of compound **4d** (1.957g, colorless oil, 10% yield). Total yield 88%.

10 **Step C.** To solid ethyl 2-acetamidomalonate **4b** (7.57g, 40mmol.) was added 6-heptenal **4d** (4.48g, 40mmol) in solution in pyridine (32mL, 10eq) over 1min. The resulting solution was cooled in a 10° bath and acetic anhydride (12mL, 3.2eq.) was added over 4min. The resulting orange solution was stirred for 3 h at RT and another portion of ethyl 2-acetamidomalonate **4b** (2.27g) was added. The resulting
15 mixture was stirred at room temperature for an extra 11h. Ice (60mL) was then added and the solution was stirred for 1.5h, then the mixture was diluted with 250mL of water and extracted with two portions of diethyl ether. The ethereal solution was washed with 1N HCl, sat. NaHCO₃, dried Na₂SO₄, concentrated and purified by flash chromatography (EtOAc 40% / hexane) to give compound **4e** (4.8g, 50% yield) as a
20 pale yellow oil.

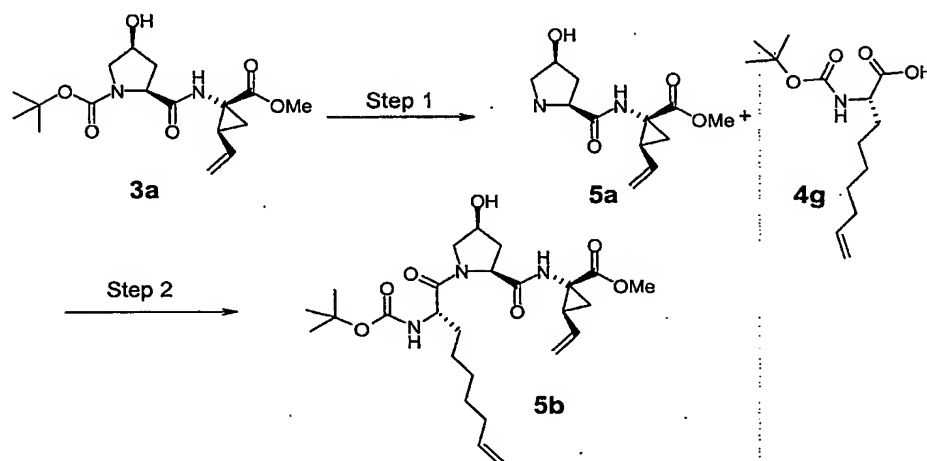
Step D. To a degassed (argon bubbling for 30 min.) solution of Z-ethyl 2-acetamido-2,8-nonadienoate **4e** (8.38g, 35mmol) in dry ethanol (70mL) was added (S,S)-Et-DUPHOS Rh(COD)OTf (51mg, (substrate/catalyst = 496). The mixture was put under 30 psi of hydrogen (after 4 vacuum-H₂ cycles) and stirred on a Parr shaker for
25 2h. The resulting mixture was evaporated to dryness to obtain the crude compound **4f**, which was used in the subsequent step without purification.

Step E. To a solution of crude (S)-ethyl 2-acetamido-8-nonenolate **4f** (7.3g, 30.3mmol) in THF (100mL), Boc₂O (13.2g, 2eq.) and DMAP (740mg, 0.2eq) were added. The reaction mixture was heated at reflux for 2.5h. Subsequently, most of
30 the THF solvent was evaporated, the crude mixture was diluted with CH₂Cl₂ and washed with 1 N HCl in order to remove the DMAP. The organic layer was further extracted with saturated aqueous NaHCO₃, dried with anhydrous Na₂SO₄ and concentrated under vacuum. The crude product was then diluted with THF (50mL) and water (30mL), LiOH.H₂O (2.54g, 2eq.) was added and the resulting mixture was
35 stirred at RT for 25 h (completion of the hydrolysis was confirmed by TLC). The

reaction mixture was concentrated under vacuum to remove most of the THF solvent and diluted with CH_2Cl_2 . The resulting solution was washed with 1 N HCl, dried with anhydrous Na_2SO_4 and concentrated under vacuum. In order to remove minor impurities and excess Boc_2O , the crude product was purified by flash chromatography (using a solvent gradient from 100% hexane – 100% EtOAc as the eluent). The titled compound **4g** was obtained in high purity as a pale yellow oil (5.82g, 71% yield). ^1H NMR (DMSO, 400 MHz): δ 7.01 (d, J = 8 Hz, 1H), 5.79 (tdd, J_t = 6.7 Hz, J_d = 17.0, 10.2 Hz, 1H), 5.00 (md, J_d = 17.0 Hz, 1H), 4.93 (md, J_d = 10.2 Hz, 1H), 3.83 (m, 1H), 2.00 (q, J = 6.9 Hz, 2H), 1.65-1.5 (m, 2H), 1.38 (s, 9H), 1.35-1.21 (m, 6H).

EXAMPLE 5

Synthesis of tripeptide **5b**



15

Step 1 : A solution of hydrogen chloride in dioxane (4N, Aldrich) was added to the Boc P2-P1 fragment **3a** (5.32g, 15.0mmol) resulting in a colorless solution. After 1h of stirring at room temperature, the solvent was evaporated and the residue placed under high vacuum for 3h which afforded the hydrochloride salt of compound **5a** as an amorphous solid which was used as such.

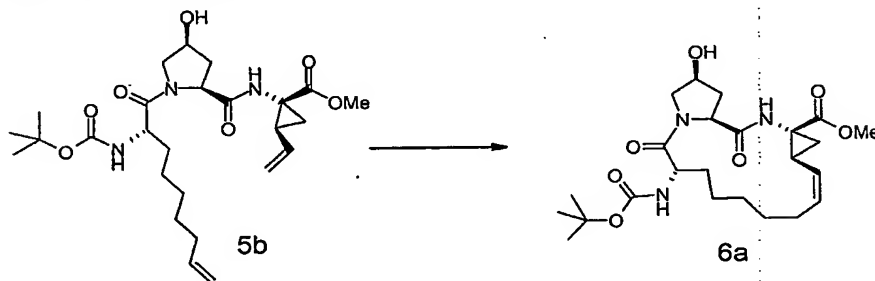
20

Step 2 : DIPEA (2.6mL, 15mmol) was added to a mixture of the above prepared P1-P2 hydrochloride (15mmol) in dry DCM (100mL) resulting in a homogeneous solution. Separately, TBTU (5.30g, 16.5mmol, 1.1equiv.) was added to a stirred solution of C9-linker **4g** (4.07g, 15.0mmol) in dry DCM (130mL) resulting in partial

dissolution of the reagent. DIPEA (2.6mL, 15mmol) was added resulting in an essentially homogeneous solution after 10 min. To this was then added the P1-P2 solution and DIPEA added until the reaction was basic (pH > 8 on wet litmus). After stirring under a nitrogen atmosphere for 5 h, the solvent was evaporated and the residue extracted with EtOAc (2 x 250mL) and washed with dilute hydrochloric acid (0.05N, 400mL), water (400mL), and saturated sodium bicarbonate (400mL). The combined organic phases were then dried over magnesium sulfate, filtered and evaporated to a yellow syrup. The crude product was chromatographed over silica gel using 6 :1 EtOAc / hexane to pure EtOAc as eluent, which afforded the desired tripeptide, diene **5b**, as a white foam (5.88g, 82%, purity >95% by HPLC).

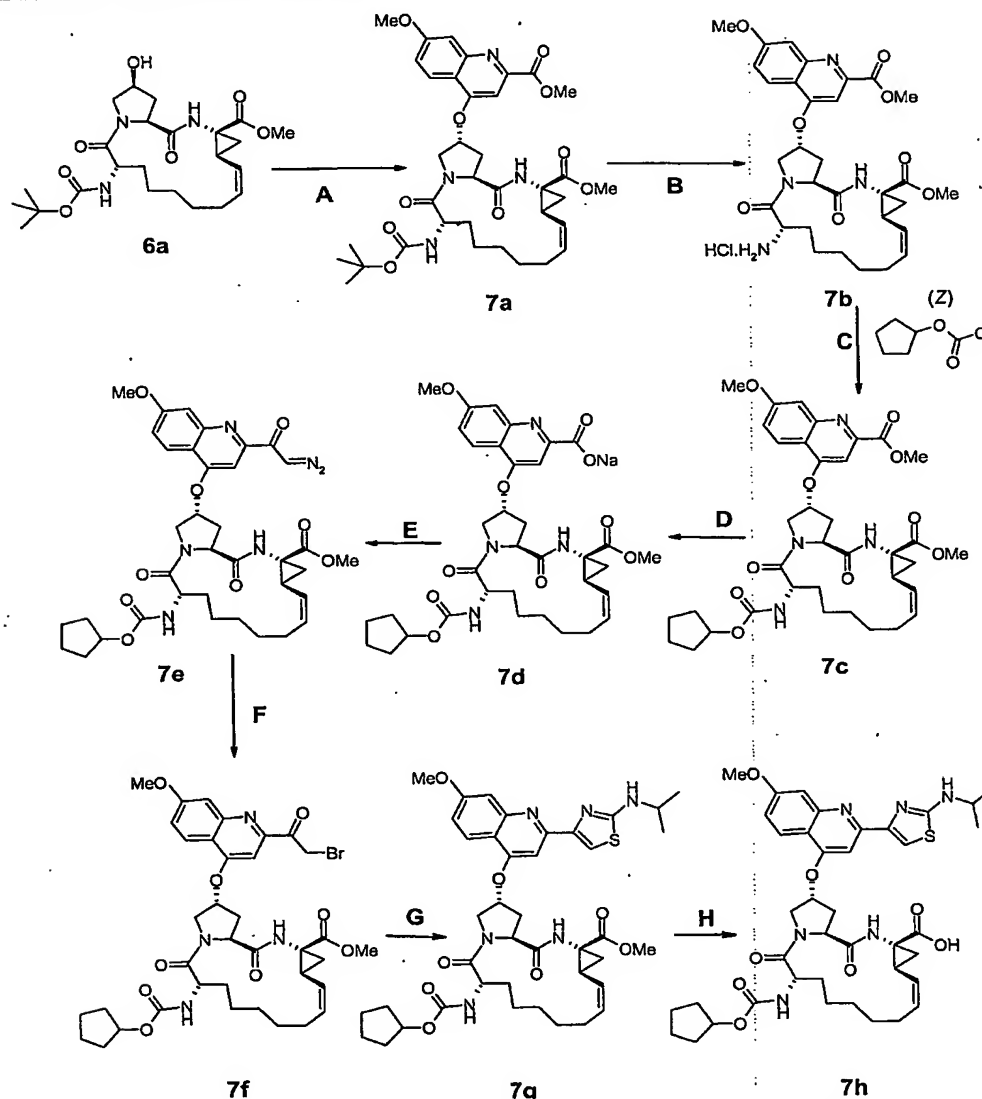
EXAMPLE 6

Synthesis of macrocyclic intermediate 6a:



A solution of diene **5b** (4.0g, 7.88mmol) in dry DCM (800mL) was deoxygenated by bubbling Ar for 2h. Hoveyda's catalyst (262mg, 0.434mmol, 5.5mol %) was then added as a solid and the reaction was refluxed under an Ar balloon. After 28h, the red-orange solution was evaporated to an amorphous solid and then purified by flash column chromatography over silica gel. The initial solvent system was 10% EtOAc in CH₂Cl₂. Once the catalyst was eluted from the column, the solvent was changed to pure EtOAc. Elution of the catalyst from the column was evident from its color. The macrocyclic product **6a** was isolated as a colorless foam which was re-dissolved in CH₂Cl₂/hexane (~1:2). Evaporation of the solvent afforded a white powder (3.362g, 89% yield).

¹H NMR (CDCl₃, 400 MHz): δ 1.20-1.50 (m, 6H), 1.43 (s, 9H), 1.53 (dd, J = 9.5 & 5.4, 1H), 1.61-1.70 (m, 1H), 1.76-1.90 (m, 2H), 2.05-2.26 (m, 4H), 2.45 (d, J = 14.3, 1H), 3.67 (s, 3H), 3.71 (d, J = 11.1, 1H), 3.90 (dd, J = 11.1 & 4.3, 1H), 4.43-4.53 (m, 2H), 4.76 (d, J = 8.6, 1H), 4.86 (bd, J = 9.8, 1H), 5.20-5.23 (m, 2H), 5.57 (dt, J = 7.0 & 9.8, 1H), 7.32 (bs, 1H).

EXAMPLE 7**Synthesis of macrocyclic tripeptide intermediate 7h:**

- 5 **Step A.** To a solution of the macrocyclic intermediate **6a** (13.05g, 27.2mmol, 1.0eq.), Ph_3P (14.28g, 54.4mmol, 2.0eq) and 2-carboxymethoxy-4-hydroxy-7-methoxyquinoline (WO 00/09543 & WO 00/09558) (6.67g, 28.6mmol, 1.05eq) in THF (450mL) at 0° , DIAD (10.75mL, 54.6mmol, 2.0eq) was added dropwise over a period of 15 min. The ice bath was then removed and the reaction mixture was stirred at RT for 3h. After the complete conversion of starting material to products,
- 10

the solvent was evaporated under vacuum, the remaining mixture diluted with EtOAc, washed with saturated NaHCO_3 (2x) and brine (1x), the organic layer was dried over anhydrous MgSO_4 , filtered and evaporated to dryness. Pure compound **7a** was obtained after flash column chromatography; the column was eluted first with
5 hexane/EtOAc (50:50), followed by CHCl_3 /EtOAc (95:5) to remove Ph_3PO and DIAD byproducts and elution of the impurities was monitored by TLC. Finally, the desired product **7a** was eluted from the column with CHCl_3 /EtOAc (70:30). Usually, the chromatography step had to be repeated 2-3 times before compound **7a** could be isolated in high purity as a white solid with an overall yield of 68% (12.8g, 99.5%
10 pure by HPLC).

Step B. To a solution of the Boc-protected intermediate **7a** (1.567g) in CH_2Cl_2 (15mL), 4N HCl in dioxane (12mL) was added and the reaction mixture was stirred at RT for 1h. [In the event that a thick gel would form half way through the reaction period, an additional 10mL CH_2Cl_2 was added.] Upon completion of the deprotection
15 the solvents were evaporated to dryness to obtain a yellow solid and a paste like material. The mixture was redissolved in approximately 5% MeOH in CH_2Cl_2 and re-evaporated to dryness under vacuum to obtain compound **7b** as a yellow solid, which was used in the next step without any purification.

Step C. To a solution of cyclopentanol (614 μL , 6.76mmol) in THF (15mL), a
20 solution of phosgene in toluene (1.93M, 5.96mL, 11.502mmol) was added dropwise and the mixture was stirred at RT for 2h to form the cyclopentyl chloroformate reagent (**z**). After that period, approximately half of the solvent was removed by evaporation under vacuum, the remaining light yellow solution was diluted by the addition of CH_2Cl_2 (5mL) and concentrated to half of its original volume, in order to
25 assure the removal of all excess phosgene. The above solution of the cyclopentyl chloroformate reagent was further diluted with THF (15mL) and added to the amine-2HCl salt **7b**. The mixture was cooled to 0° in an ice bath, the pH was adjusted to ~8.5-9 with the addition of Et_3N (added dropwise) and the reaction mixture was stirred at 0° for 1h. After that period, the mixture was diluted with EtOAc, washed
30 with water (1x), saturated NaHCO_3 (2x), H_2O (2x) and brine (1x). The organic layer was dried over anhydrous MgSO_4 , filtered and evaporated under vacuum to obtain a yellow-amber foam. Dimethyl ester **7c** was obtained as a white foam after purification by flash column chromatography (using a solvent gradient from 30% hexane to 20% hexane in EtOAc as the eluent) in 80% yield (1.27g) and >93%
35 purity.

Step D. The dimethyl ester **7c** (1.17g) was dissolved in a mixture of THF/MeOH/H₂O (20mL, 2:1:1 ratio), and an aqueous solution of NaOH (1.8mL, 1N, 1eq.) was added. The reaction mixture was stirred at RT for 1h before it was evaporated to dryness to obtain the sodium salt **7d** as a white solid (~1.66mmol).

5 Compound **7d** was used in the next step without purification.

Step E. The crude sodium salt **7d** (1.66mmol) was dissolved in THF (17mL), Et₃N was added and the mixture was cooled to 0° in an ice bath. Isobutylchloroformate (322 µL, 2.5mmol) was added dropwise and the mixture was stirred at 0° for 75min. After that period, diazomethane (15mL) was added and stirring was continued at 0°
10 for 30min and then at RT for an additional 1h. Most of the solvent was evaporated to dryness under vacuum, the remaining mixture was diluted with EtOAc, washed with saturated NaHCO₃ (2x), H₂O (2x) and brine (1x), dried over anhydrous MgSO₄, filtered and evaporated to dryness to obtain compound **7e** as a light yellow foam (1.2g, ~1.66mmol). The diazoketone intermediate **7e** was used in the next step
15 without purification.

Step F. The diazoketone **7e** (1.2g, 1.66mmol) dissolved in THF (17mL) was cooled to 0° in an ice bath. A solution of aqueous HBr (48%, 1.24mL) was added dropwise and the reaction mixture was stirred at 0° for 1h. The mixture was then diluted with EtOAc, wash with saturated NaHCO₃ (2x), H₂O (2x) and brine (1x), the organic layer
20 was dried over anhydrous MgSO₄, filtered and evaporated to dryness to obtain the α-bromoketone intermediate **7f** as a light yellow foam (~1.657mmol).

Step G. To a solution of the bromoketone **7f** (1.66mmol) in isopropanol (50mL), isopropylthiourea (392mg, 3.32mmol) was added and the reaction mixture was placed in a preheated oil bath at 75° where it was allowed to stir for 1h. The
25 isopropanol was then removed under vacuum and the product dissolved in EtOAc (250mL). The solution was washed with saturated NaHCO₃ and brine, the organic layer was dried over anhydrous Na₂SO₄, filtered and evaporated to afford the crude product **7g** (1.35g) as a cream colored solid. The crude product was purified by flash chromatography in silica gel (1:1hexane/EtOAc) to afford 860mg of an off-white
30 solid (66% yield over 4 steps).

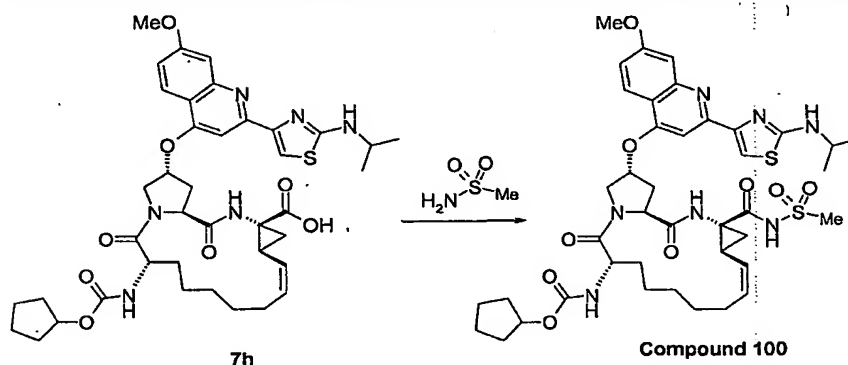
Step H. The methyl ester **7g** (860mg, 1.1mmol) was dissolved in a solution of THF/MeOH/H₂O(2:11,24mL) and saponified with LiOH. H₂O(369mg, 8.8mmol). The hydrolysis reaction was carried out over 12h at RT. Thereafter, the solution was evaporated to dryness to give an off-white paste. The paste was diluted with EtOAc
35 and brine. The mixture was adjusted to pH 6 with 1N HCl. The EtOAc layer was

separated and the aqueous layer was extracted twice with EtOAc. The combined EtOAc extracts were washed with deionized water (2X) and brine (1X), dried (MgSO_4), and evaporated to afford the cyclic tripeptide intermediate **7h** as a yellow solid (818mg; 97% yield).

- 5 ^1H NMR (400 MHz, DMSO-d_6): δ 8.59(s, 1H), 8.03(d, $J=9\text{Hz}$, 1H), 7.43(s, 2H), 7.28(d, $J=2\text{Hz}$, 1H), 7.23 (d, $J=6.7\text{Hz}$, 1H), 7.02 (dd, $J=1.9, 8.9\text{ Hz}$, 1H), 5.54-5.41 (m, 2H), 5.28 (bdd, $J=9.2, 9.2\text{ Hz}$, 1H), 4.73-4.64 (m, 1H), 4.54-4.42 (m, 2H), 4.17-4.08 (m, 1H), 3.96-3.91 (m, 1H), 3.90 (s, 3H), 3.86-3.76 (m, 1H), 2.60-2.44 (m, 1H), 2.43-2.31 (m, 1H), 2.23-2.13 (m, 1H), 1.82-1.28 (m, 20H), 1.26 (d, $J=1.3\text{ Hz}$, 3M), 1.24 (d, $J=1.3\text{Hz}$, 3H).
- 10

EXAMPLE 8

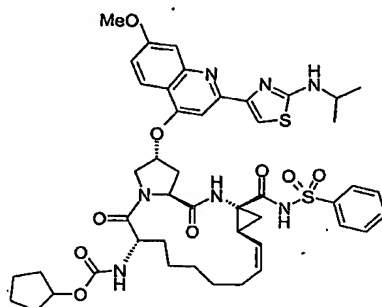
Preparation of compound **100** (Compound of formula **I** wherein R^1 is methyl)



- 15 The cyclic tripeptide intermediate **7h** (20mg, 0.026mmol) was combined with HATU (12mg, 0.031mmol) in anhydrous DMF (4mL). The solution was stirred at R.T. before DIPEA (23 μL , 0.13mmol) was added dropwise over ca. 1 min. The mixture was stirred for 40 min. at R.T. and analyzed by analytical HPLC for the formation of the activated ester. A solution of methanesulfonamide (12.4mg, 0.13mmol), DMAP
- 20 (14.3mg, 0.12mmol) and DBU (15.5 μL , 0.10mmol) were added in DMF (1mL). The reaction mixture was stirred 36hr at R.T. before being concentrated. The residue was reconstituted in DMSO and purified by preparative HPLC. Lyophilization of pure fractions gave the sulfonamide derivative **100** (7.95mg, 36%) as a bright yellow amorphous solid.
- 25 MS (electrospray): 852.5 ($\text{M} + \text{H}$) $^+$, and 850.5 ($\text{M} - \text{H}$) $^-$.
 RP-HPLC: Rt = 6.6 minutes (homogeneity = 100%).

EXAMPLE 9

Preparation of compound 101 (Compound of formula I wherein R¹ is phenyl)



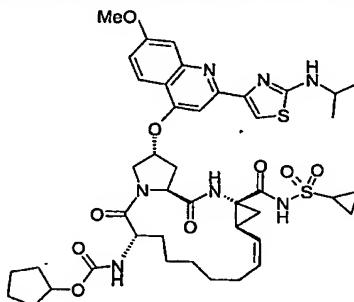
Compound 101

Using the same protocol as described in example 8 except using

- 5 benzenesulfonamide instead of methanesulfonamide, the phenyl analog **101** was prepared as a bright yellow amorphous solid in 27% yield.
MS (electrospray); 914.5 (M + H)⁺, and 912.5 (M - H)⁻.
RP-HPLC: Rt = 7.2 minutes (homogeneity = 100 %).

EXAMPLE 10

Preparation of compound 102 (Compound of formula I wherein R¹ is cyclopropyl)



Compound 102

- The cyclic tripeptide intermediate **7h** (20 mg, 0.026 mmol) was combined with HATU (29.5 mg, 0.077 mmol) in anhydrous DMF (4 mL). The solution was stirred at R.T.
- 15 before DIPEA (23 μ L, 0.13 mmol) was added dropwise over ca. 1 min. The mixture was stirred for 45 minutes at R.T. and analyzed by analytical HPLC for the formation of the activated ester at 6.6 minutes.
- Next, a solution of the cyclopropanesulfonamide (12.6 mg, 0.104 mmol), DMAP (14.3 mg, 0.117 mmol) was added all at once in DMF (1 mL). After 2 hr, DBU (15.5
- 20 mL, 0.104 mmol) was added and the reaction mixture stirred 20 h at R.T. before

being concentrated. The reaction was reconstituted in DMSO and purified by preparative HPLC. Lyophilization of pure fractions gave the sulfonamide derivative 102 as a yellow solid (6.5 mg, 28.5%).

MS (electrospray); 878.4 (M + H)⁺, and 876.4 (M - H)⁻.

5 RP-HPLC: Rt = 6.9 minutes (homogeneity = 100 %).

Biological assays

NS3-NS4A protease assay

The enzymatic assay used to evaluate the present compounds is described in WO
10 00/09543 and WO 00/59929.

Cell Based HCV RNA Replication Assay

The cell-based HCV RNA replication assay used to evaluate the present compounds is described in detail in WO03/010141.

15

Some of the compounds of this invention were evaluated the preceding enzymatic and cell based assays and were found to be highly active. More specifically, the compounds had IC₅₀'s below 0.1 μ M in the NS3-NS4A protease assay, and EC₅₀'s below 0.5 μ M in the cell based HCV RNA replication assay.

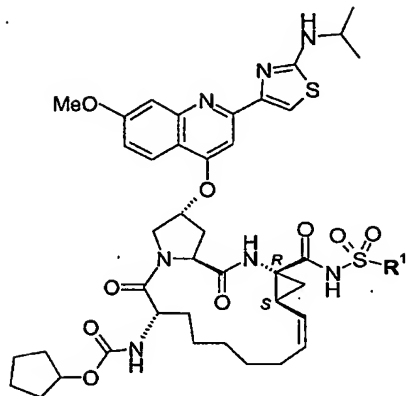
20

Specificity assays

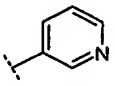
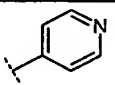
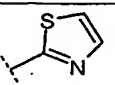
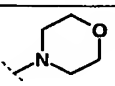
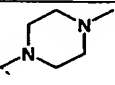
The specificity assays used to evaluate the selectivity of this compound are described in WO 00/09543.

25 When the compounds were evaluated in the specificity assays, the compounds of formula I were found to be selective in that they do not show significant inhibition in the Human Leukocyte Elastase and Cathepsin B assays.

Table of compounds



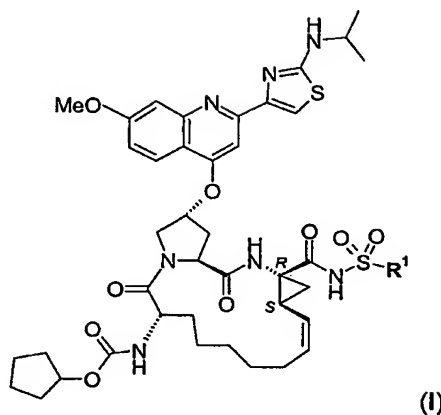
Compound #	R ¹
100	methyl
101	phenyl
102	cyclopropyl
103	ethyl
104	<i>i</i> -propyl
105	<i>n</i> -propyl
106	<i>n</i> -butyl
107	<i>sec</i> -butyl
108	<i>n</i> -pentyl
109	2-Me-butyl
110	3-Me-butyl
111	cyclobutyl
112	cyclopentyl
113	-CH ₂ -cyclopropyl
114	-CH ₂ -CH ₂ -cyclohexyl
115	CCl ₃
116	CF ₃
117	2-fluorophenyl
118	4-methylphenyl
119	

120	
121	
122	
123	
124	

CLAIMS

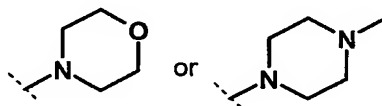
WHAT IS CLAIMED IS:

1. A compound of formula (I)

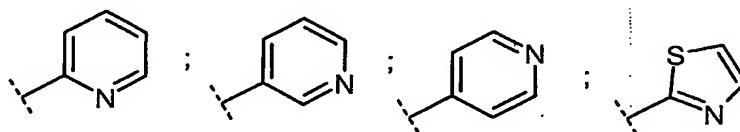


wherein R^1 is (C_{1-6}) alkyl, (C_{3-7}) cycloalkyl, $\{(C_{1-6})$ alkyl- (C_{3-7}) cycloalkyl $\}$ or Het, which are all optionally substituted from 1 to 3 times with halo, cyano, nitro, $O-(C_{1-6})$ alkyl, amido, amino or phenyl; or R^1 is C_6 or C_{10} aryl which is optionally substituted from 1 to 3 times with halo, cyano, nitro, (C_{1-6}) alkyl, $O-(C_{1-6})$ alkyl, amido, amino or phenyl; or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1, wherein said Het is:



or a heteroaryl selected from:



said heteroaryl being optionally substituted with C_{1-6} alkyl.

3. A compound according to claim 1, wherein R^1 is (C_{1-6}) alkyl, (C_{3-7}) cycloalkyl or $\{(C_{1-6})$ alkyl- (C_{3-7}) cycloalkyl $\}$, which are all optionally substituted from 1

to 3 times with halo, cyano, nitro, O-(C₁₋₆)alkyl, amido, amino or phenyl; or R¹ is C₆ or C₁₀ aryl which is optionally substituted from 1 to 3 times with halo, cyano, nitro, (C₁₋₆)alkyl, O-(C₁₋₆)alkyl, amido, amino or phenyl; or a pharmaceutically acceptable salt thereof.

4. A compound according to claim 3, wherein R¹ is (C₁₋₆)alkyl, (C₃₋₆)cycloalkyl or {(C₁₋₆)alkyl-(C₃₋₆)cycloalkyl}, which are all optionally substituted from 1 to 3 times with halo, nitro or O-(C₁₋₆)alkyl, or phenyl which is optionally substituted from 1 to 3 times with halo, nitro, (C₁₋₆)alkyl or O-(C₁₋₆)alkyl.
5. A compound according to claim 4, wherein R¹ is methyl, ethyl, *n*-propyl, *i*-propyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclopropylmethyl, cyclohexylethyl, CCl₃, CF₃, phenyl, 2-fluorophenyl, or 4-methylphenyl.
6. A compound according to claim 5 wherein R¹ is methyl, cyclopropyl, CF₃ or phenyl.
7. A compound according to claim 6 wherein R¹ is methyl.
8. A compound according to claim 6 wherein R¹ is cyclopropyl.
9. A compound according to claim 6 wherein R¹ is phenyl.
10. A pharmaceutical composition comprising an anti-hepatitis C virally effective amount of a compound of formula I according to claim 1, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent.
11. A pharmaceutical composition according to claim 10, further comprising a therapeutically effective amount α -interferon
12. A pharmaceutical composition according to claim 10, further comprising a therapeutically effective amount of pegylated α -interferon.
13. A pharmaceutical composition according to claim 10, further comprising a

therapeutically effective amount of ribavirin.

14. A pharmaceutical composition according to claim 11, further comprising a therapeutically effective amount of ribavirin.
15. Use of an anti-hepatitis C virally effective amount of a compound of formula I according to claim 1, or a pharmaceutically acceptable salt thereof for the preparation of a medicament for treating or preventing a hepatitis C viral infection in a mammal.
16. Use of a hepatitis C viral NS3 protease inhibiting amount of a compound of formula I according to claim 1, or a pharmaceutically acceptable salt thereof for the preparation of a composition for inhibiting the replication of hepatitis C virus.
17. Use of a compound of formula I according to any one of claims 1 to 9 for the manufacture of a medicament for the treatment of hepatitis C viral infection.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 03/01604

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C07K5/08 A61K38/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

CHEM ABS Data, WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 03 053349 A (SQUIBB BRISTOL MYERS CO) 3 July 2003 (2003-07-03) cited in the application The whole document; see especially cmp nrs. 34,122,131,142,149	1,3-8, 10-17
Y	WO 00 59929 A (BOEHRINGER INGELHEIM CA LTD ;GOUDREAU NATHALIE (CA); GHIRO ELISE () 12 October 2000 (2000-10-12) Claims 1,52;compound nr.822; examples 34c	1,3-17
Y	WO 02 060926 A (SQUIBB BRISTOL MYERS CO) 8 August 2002 (2002-08-08) the whole document	1,3-17
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

10 February 2004

Date of mailing of the international search report

24/02/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Groenendijk, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01604

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, Y	WO 03 064455 A (BOEHRINGER INGELHEIM CA LTD ; GORYS VIDA J (CA); LLINAS-BRUNET MONT) 7 August 2003 (2003-08-07) claims 1-4, 10-23; cpd. 103 -----	1, 3-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 03/01604

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 03053349	A	03-07-2003	WO 03053349 A2	03-07-2003
WO 0059929	A	12-10-2000	AU 3548000 A	23-10-2000
			BG 105970 A	31-05-2002
			BR 0009599 A	15-01-2002
			WO 0059929 A1	12-10-2000
			CA 2367127 A1	12-10-2000
			CN 1346365 T	24-04-2002
			CZ 20013467 A3	12-06-2002
			EE 200100516 A	16-12-2002
			EP 1169339 A1	09-01-2002
			HR 20010720 A1	31-12-2002
			HU 0300121 A2	28-06-2003
			ID 30459 A	06-12-2001
			JP 2002542160 T	10-12-2002
			NO 20014857 A	31-10-2001
			PL 350855 A1	10-02-2003
			SK 14072001 A3	05-03-2002
			TR 200102878 T2	21-01-2002
			US 2004002448 A1	01-01-2004
			US 6608027 B1	19-08-2003
WO 02060926	A	08-08-2002	CA 2429359 A1	08-08-2002
			EP 1337550 A2	27-08-2003
			US 2002111313 A1	15-08-2002
			WO 02060926 A2	08-08-2002
WO 03064455	A	07-08-2003	CA 2369711 A1	30-07-2003
			WO 03064455 A2	07-08-2003
			US 2003181363 A1	25-09-2003
			US 2003224977 A1	04-12-2003